Novel starch thermoplastic/Bioglass® composites: Mechanical properties, degradation behavior and *in-vitro* bioactivity

I. B. LEONOR*, R. A. SOUSA, A. M. CUNHA, R. L. REIS
Department of Polymer Engineering, University of Minho, Campus de Azurem, 4800-058 Guimarães, Portugal

Z. P. ZHONG, D. GREENSPAN
US Biomaterials Corporation, One Progress Blvd. Box 23, Alachua, Florida 32615, USA
E-mail: belinha@dep.uminho.pt

The present research aims to evaluate the possibility of creating new degradable, stiff and highly bioactive composites based on a biodegradable thermoplastic starch-based polymeric blend and a Bioglass® filler. Such combination should allow for the development of bioactive and degradable composites with a great potential for a range of temporary applications. A blend of starch with ethylene–vinyl alcohol copolymer (SEVA-C) was reinforced with a 45S5 Bioglass® powder presenting a granulometric distribution between 38 and 53 μm. Composites with 10 and 40 wt % of 45S5 Bioglass® were compounded by twin-screw extrusion (TSE) and subsequently injection molded under optimized conditions. The mechanical properties of the composites were evaluated by tensile testing, and their bioactivity assessed by immersion in a simulated body fluid (SBF) for different periods of time. The biodegradability of these composites was also monitored after several immersion periods in an isotonic saline solution. The tensile tests results obtained indicated that SEVA-C/Bioglass® composites present a slightly higher stiffness and strength (a modulus of 3.8 GPa and UTS of 38.6 MPa) than previously developed SEVA-C/Hydroxylapatite (HA) composites. The bioactivity of SEVA-C composites becomes relevant for 45S5 amounts of only 10 wt %. This was observed by scanning electron microscopy (SEM) and confirmed for immersion periods up to 30 days by both thin-film X-ray diffraction (TF-XRD) (where HA typical peaks are clearly observed) and induced coupled plasma emission (ICP) spectroscopy used to follow the elemental composition of the SBF as function of time. Additionally, it was observed that the composites are biodegradable being the results correlated with the correspondent materials composition.

1. Introduction

With the discovery of 45S5 Bioglass® by Hench *et al*. [1] it was possible to have a glass with a high level of bioactivity that elicit specific physiological responses at interfaces with tissues [2]. The primary advantage of this type of glass is its ability to bond to bone and soft tissues very quickly [3, 4], which it is attributed to its high level of surface reactivity [5–7]. However, one common problem of glasses, as well as of ceramics, is their brittle nature, which is an obstacle for their use in load-bearing clinical applications [8, 9]. In spite of that, Bonfield *et al*. [10] developed hydroxyapatite (HA) reinforced high density polyethylene (HDPE) composites designated as HAPEX™, that have been successfully applied in the medical field during the last years [11, 12]. These materials were designed to have specific bone-analog mechanical properties and bone-bonding properties due the osteoconductive character of HA [13, 14]. Furthermore, the idea of combining a bioactive ceramic with a fracture tough phase, such as a polymer, to produce a composite with mechanical properties analogous to those of cortical bone and a bioactive character [4] has been explored in several works in the last few years, including the development of polyethylene/Bioglass® composites [15–17].

However, the mechanical properties of these systems are still behind the envisaged targets that allow for their use in high load bearing applications. Furthermore, the inert character of the polymer matrix is not adequate to temporary applications. In order to achieve such objectives, composite systems of several biodegradable polymers with bioactive ceramics [18–21] have been

*Author to whom all correspondence should be addressed.

studied in the past few years. Devices obtained with biodegradable matrix composites present great advantages, as they do not have to be removed in a second surgery [22–24]. In fact, an adequate degradation behavior of the matrix allows for a gradual load transfer to the healing tissues, avoiding the traditional stress shielding effects associated with the use of very stiff materials [22–24]. In the last few years, starch-based polymeric blends have been proposed as alternative biomaterials for such type of applications. These materials combine a degradable behavior with an interesting combination of mechanical properties. They are also able to exhibit a bioactive character through the incorporation of bone-like inorganic fillers, such as HA or bioactive glasses and glass–ceramics [25–29] powders. Additionally, it has been shown [25, 30–32] that these materials can comply with the biocompatibility requirements of a biomaterial, as defined in international standards, which is not typical of biodegradable systems.

The present study evaluates the possibility of creating new stiff and bioactive composites composed by a biodegradable starch based blend reinforced with a bioactive glass (Bioglass®) filler. The main objective of the work was to produce biodegradable composites exhibiting a good mechanical performance and a clear bioactive behavior.

2. Materials and methods

2.1. Materials

The study matrix was a thermoplastic blend of starch with poly (ethylene–vinyl alcohol) copolymer (50/50 wt %), designated as SEVA-C, supplied by Novamont, Italy, with a melt flow index (MFI) value of 0.71 g/10 min (170 °C, 49 N).

The select reinforcement filler was a 45S5 Bioglass® powder with a composition of 45 SiO_2, 24.5 CaO, 24.5 Na_2O and 6.0 P_2O_5 in wt %, supplied by US Biomaterials Corp., USA. The powder used, exhibited a granulometric distribution between 38 and 53 μm, as determined by laser scattering analysis using a model Coulter LS 100 particle size analyzer.

Two types of composites were produced, corresponding to 10% and 40% weight incorporation of this filler in the polymeric matrix. These two compounds will be referred to as 10-SEVA-C/Bioglass® and 40-SEVA-C/Bioglass®, respectively.

2.2. Extrusion compounding

The compounds were produced in a Leistriz AG-LSM 36/25D modular co-rotating twin screw extruder (TSE). The configuration of the screw was designed in order to promote the best possible interaction between the filler and the polymer and to minimize any eventual thermal degradation of the starch-based blend that arise from the high shear rates imposed to the melt. The presence of Bioglass® particles increases the viscosity of the melt, leading to high viscous heat dissipation and, consequently, requiring special caution to minimize thermal degradation of the highly thermo-sensible matrix. A screw speed of 40 to 60 rpm and a temperature profile (from feeding to die zone) of 150 to 175 °C were used during compounding. The cooling of the extrudate was performed in air. No quenching was applied.

The SEVA-C/Bioglass® composites were injection molded from these compounds, under optimized conditions, in a Klockner-Ferromatik Desma FM20 machine into small dumb-bell ASTM tensile samples with a rectangular cross-section ($2 \times 4\,mm^2$).

2.3. Mechanical testing

The composites were tensile tested in an Instron 4505 machine, using a resistive extensometer, in order to determine the secant modulus at 1% strain ($E_{1\%}$), the ultimate tensile strength (UTS) and the strain at break ($\varepsilon_{r\%}$). The tests were conducted in a controlled environment (23 °C and 55% relative humidity – RH) using a cross-head speed of 5 mm/min (8.3×10^{-5} m/s) until 1% strain and then increased to 50 mm/min (8.3×10^{-4} m/s) until fracture. The fracture surfaces were examined by scanning electron microscopy (SEM) in a Leica Cambridge S360 microscope.

2.4. *In vitro* bioactivity tests

Standard *in vitro* bioactivity tests were carried out to evaluate the formation *per si* (or not) of an apatite layer on the surface of the composites, considered as an indicator of the *in vivo* bioactivity of the composite. In order to study the bioactivity, the samples were soaked in simulated body fluid (SBF) at 37 °C and pH = 7.35 for several periods of time up to 30 days. The SBF has a composition similar to human blood plasma and has been extensively used for *in vitro* bioactivity tests [33]. At the end of each immersion period, the samples were rinsed with distilled water and dried in a controled environment (23 °C and 55% RH).

2.5. Degradation tests

The degradation behavior of the composites was assessed for several prefixed aging periods in a simulated physiological solution (isotonic saline solution, 0.154 M sodium chloride solution) at 37 °C using two sample batches for each test condition. After being removed from the solution, one batch of samples was dried in an oven for 48 h at 70 °C, in order to determine the respective weight loss. The other batch was stored in a room under controled atmosphere (23 °C and 55% RH) for two weeks, in order to stabilize the moisture content. After stabilization, the samples for each test period were tensile tested, as previously described, and their mechanical properties accessed as a function of the *in vitro* aging time.

2.6. Surface analysis

The surface morphology and the correspondent calcium–phosphorus (Ca/P) ratios of any film detected were analyzed (before and after immersion in SBF) using scanning electron microscopy and energy dispersive spectroscopy (SEM/EDS). The calcium/phosphorous ratio (Ca/P) of the films was determined using well-stabilized sub-routines for EDS semi-quantitative ana-

TABLE I Tensile properties of conventionally injection molded SEVA-C and SEVA-C/Bioglass® composites

Filler (wt %)	Granulometric distributions* (μm)	$E_{1\%}$ (GPa)	UTS (MPa)	ε_r (%)
0	—	1.86 ± 0.12	42.3 ± 2.7	14.7 ± 6.5
10% Bioglass®	53–38	3.54 ± 0.24	50.7 ± 1.1	2.5 ± 0.1
40% Bioglass®	53–38	3.77 ± 0.26	38.6 ± 3.1	1.8 ± 0.2
10% HA	7	3.47 ± 0.15	47.5 ± 3.4	1.9 ± 0.2
40% HA	7	6.56 ± 0.38	34.3 ± 0.7	1.2 ± 0.5

* 90% of the particles (below this size in case of HA).

lysis. Thin-film X-ray diffraction (TF-XRD – incidence angle of 1°) was used to characterize the crystalline/amorphous nature of the films and to identify any crystalline phases present after immersion in SBF (results were compared to non-immersed controls).

2.7. Solution analysis

The solutions of the bioactivity tests were analyzed by induced coupled plasma emission (ICP) spectroscopy in order to determine the evolution of the elemental concentration of Ca, P, Si and Na ions as function of immersion time. These results are very useful to complement the data obtained by SEM/EDS and TF-XRD.

3. Results and discussion

3.1. Mechanical results

The tensile test results for SEVA-C/Bioglass® composites and SEVA-C/HA (included as reference for comparison purposes) are presented in Table I. These data refer to specimens obtained in optimized processing conditions. As expected, and already reported in previous works [26, 27], the addition of stiff fillers (as HA or other types of glasses) to a starch-based blend improves significantly respective modulus. The 10-SEVA-C/Bioglass® composite present slightly higher values of stiffness and strength than the HA filled materials. However, for a reinforcement amount of 40 wt %, SEVA-C/Bioglass® composites present lower stiffness values than the HA reinforced composites (although they exhibit a higher strength). Nevertheless, it is very important to note that HA particles present a much smaller average particle size – around 7 μm – that consequently leads to a smaller stress concentration effect, and a much better dispersion of the filler particles, when compared to the larger Bioglass® particles. In fact, the mechanical properties obtained with this large particle size reinforcement (a modulus of 3.8 GPa and an UTS of 38.6 MPa) are very promising and better than those obtained for a HDPE-based matrix [15].

The final mechanical performance of particulate filler composite depends on the granulometry of the filler, its volume amount, and the respective processing conditions. An increase in the filler amount leads to the achievement of a higher modulus, while the UTS and the strain at break are decreased. This behavior is typical of polymeric matrix composites reinforced with ceramic/glass particles, as it has been shown in several other studies [26, 27, 29]. The observed decrease of tensile strength may be associated with a poor interfacial interaction between the filler and the matrix, which limits the load transfer during the mechanical solicitation of the composite. The observation of the tensile fracture surfaces of the composites allows for the evaluation of the apparent ductility of the developed materials, as well as the quality of the polymer/reinforcement interaction. Fig. 1(a) and (b) presents the typical tensile fracture surfaces of composites of SEVA-C with 10 and 40 wt % 45S5, respectively. The observation of these figures suggests a poor interfacial interaction between the filler and the matrix, which is in agreement with the relatively low values of strength reported.

One way of improving the interfacial interaction between the composite phases would be by means of using coupling agents, which may establish ''linking bridges'' between the filler and the matrix, resulting in a higher degree of filler/matrix interaction and an improved mechanical performance [34]. This approach should be tested in future studies. Nevertheless, the obtained tensile test results and the tensile fracture surfaces of these composites indicate that the combined

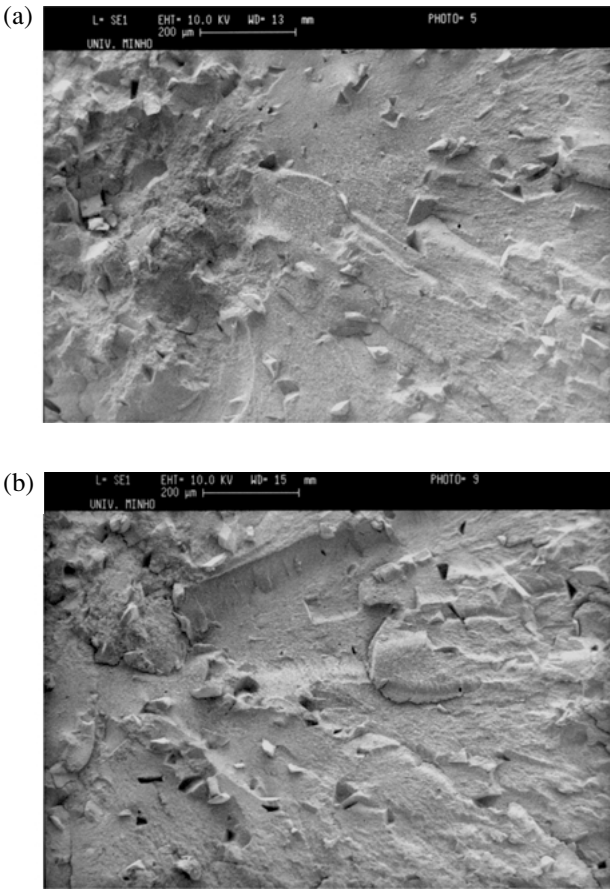

Figure 1 SEM micrographs of tensile fracture surfaces of SEVA-C/Bioglass® composites (a) 10 wt % 45S5 and (b) 40 wt % 45S5.

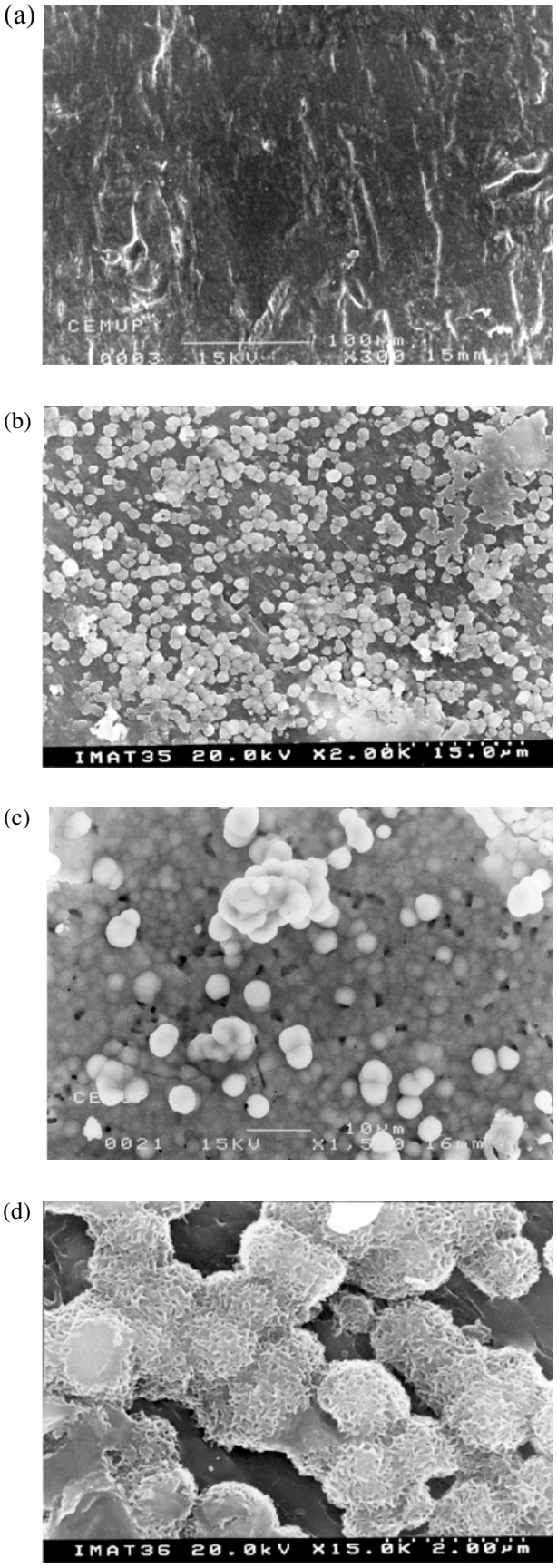

Figure 2 SEM micrographs showing the formation of an apatite layer on the surface of SEVA-C + 10 wt % 45S5 composite after 0 (a), 7 (b), and 30 days (c) immersion in SBF at 37 °C. Magnification (d) showing a detail of the structure presented in (b).

use of TSE with optimized routines in injection molding might allow for the production of biodegradable composites exhibiting an homogeneous distribution of the filler and attractive values of stiffness and strength.

3.2. Bioactivity tests

The bioactivity tests showed that for all filler amounts the Bioglass®/SEVA-C composites developed an apatite layer on its surface after immersion in a SBF solution, which confirms the bioactive character of the composite (see Fig. 2), and its expected *in vivo* bone-bonding behavior.

These results show that the Bioglass® filler is highly bioactive, since for only 10 wt % it is possible to obtain an apatite layer only after seven days of immersion (Fig. 2(b)). In fact, for SEVA-C/HA composites, an amount of at least 30 wt %. HA is required to observe the same type of behavior [25, 35]. The water-uptake ability of the polymer, that gives access to the inner Bioglass® particles, associated to the higher reactivity of this filler are the main responsible for the observed bioactivity. It might be concluded that a weight amount of 10% is enough to confer a bioactive character to SEVA-C/Bioglass® composites. For longer immersion periods (up to 30 days), the thickness and the density of the apatite layers increased (see Fig. 2(c)). This effect is even more pronounced for 40 wt % Bioglass® composite. These results are attributed to the higher quantity and more uniform distribution of the Bioglass® particles in the composites. Results not presented here have shown that the bioactive behavior of this system is further increased with the use of Bioglass® powders with smaller average particle sizes. EDS analysis of the films showed Ca/P ratios in the 1.5–1.8 range, i.e., between tricalcium phosphate (TCP) and HA.

The TF-XRD analysis of these composites obtained after different immersion periods in SBF solution (using non-immersed samples as controls), evidenced the existence of the characteristic main peaks of HA. This fact was confirmed by the matching of the XRD spectra with the standard pattern of HA (JCPDS 9-432). For longer immersion times, it was possible to observe a gradual increase in the intensity of these apatite like peaks, which corresponds to the growth of an apatite like layer on the composite surface (Fig. 3). For all cases, it was clear the partial amorphous nature of the formed Ca–P layer that resembles typical human bone apatite.

The fast formation of the Ca–P layer in the SEVA-C/Bioglass® composites results from the high water-uptake capability of the matrix, which favors the dissolution of the bioactive glass particles. As consequence of the dissolution of the filler, there is an increase of Si and Na

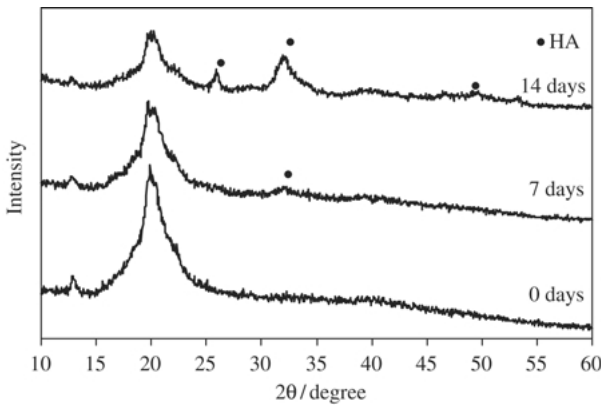

Figure 3 XRD patterns of film formed on SEVA-C + 10 wt % 45S5 composite after 0, 7 and 14 days, immersion in SBF at 37 °C.

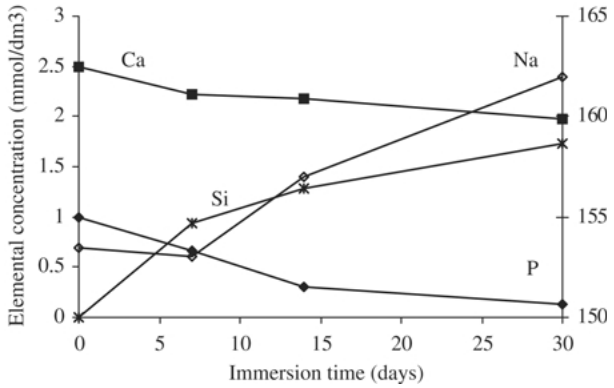

Figure 4 Evolution of Ca, P, Si and Na elemental concentrations (ICP results) in the SBF solution as a function of the immersion time for SEVA-C + 10 wt % 45S5 composite.

concentration (as it is shown in Fig. 4), which provides favorable sites for the formation of Ca–P nuclei during the initial period of immersion. During the growth of the Ca–P nuclei, the consumption of the Ca and P ions from the surrounding fluid occurs, resulting in the observed decrease of the Ca and P concentration in the solution. The growth of the Ca–P nuclei leads to the formation of an apatite layer on the surface of the SEVA-C/Bioglass® composite.

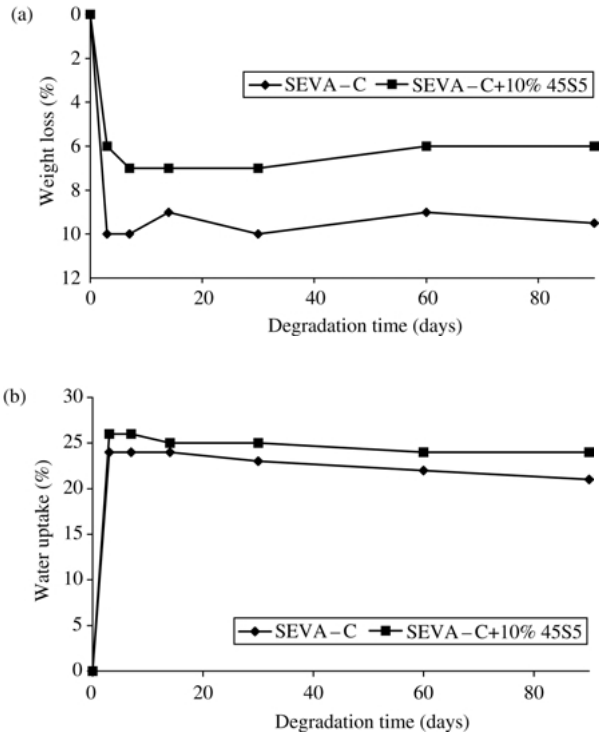

Figure 5 Evolution of weight loss (a) and water-uptake (b) in 0.154 M NaCl solution as a function of the immersion time for 0 and 10 wt % 45S5 reinforcement.

3.3. Degradation tests

On the degradation experiments only composites with 10 wt % 45S5 were studied. As mentioned in the previous section (3.2), this percentage is high enough to confer a bioactive character to SEVA-C/Bioglass® composites. The degradation behavior of the several developed materials is plotted in Fig. 5 (dry weight loss vs. degradation time).

It was possible to observe that the filled materials degraded slower than the unfilled SEVA-C due to the different degradation rate of the glass particles. In none of the cases, it was possible to observe signs of preferential attack at the polymer/filler interface that would be an indication of the presence of thermally degraded polymer. This fact is particularly relevant since SEVA-C is a highly thermo sensible blend that tends to degrade easily during processing. On the contrary, the water-uptake of the composite materials is higher than the observed for the unfilled SEVA-C. This fact results from the presence of the Bioglass® particles that limits the final orientation level of the polymer matrix, which generates a composite with a poorly orientated polymer matrix with a high amorphous content exhibiting a high water-uptake capability. Some water absorption might also be occurring preferentially at the polymer/glass interfaces.

The results of tensile tests for the degraded samples (see Table II) show that the UTS and the strain at break of the composites tend to decrease as a function of the immersion time. This deterioration of the mechanical properties is typical of biodegradable systems reinforced with particulate fillers. However, the decrease of the strain at break is less pronounced than for the unfilled SEVA-C.

Within the tested period, the modulus tends to increase as function of the degradation time. This type of behavior has been already observed before for SEVA-C composites [25, 26], and results from the leaching of processing additives, namely plasticizers during the first stages of the degradation. In a second stage, there is a matrix lubrication effect that enhances the ductility of the composite. Only after this period (for a degradation time of 60 to 90 days) the chemical degradation of the polymer occurs, with the occurrence of chain breakage, which leads to the decrease of the measured stiffness. This type of behavior is desirable for the development of polymeric-based composites exhibiting controlled mechanical and chemical properties that are able to withstand physiological loads during the first stages of implantation. Such compromise of properties can be very useful in temporary applications requiring bone fixation plates or/and pins with controled degradation behavior.

TABLE II Results of tensile tests for SEVA-C matrix and SEVA-C/Bioglass® composites after several degradation times

Bioglass® amount (wt %)	Degradation time (days)	$E_{1\%}$ (GPa)	UTS (MPa)	ε_r (%)
0	3	3.82 ± 0.42	61.7 ± 1.34	9.41 ± 1.20
0	7	3.41 ± 1.34	61.9 ± 0.51	8.28 ± 0.60
0	14	5.57 ± 2.52	61.8 ± 0.27	6.69 ± 0.24
0	30	6.04 ± 2.89	62.4 ± 0.37	6.15 ± 0.19
0	60	5.85 ± 2.05	60.6 ± 0.22	5.86 ± 0.21
0	90	5.48 ± 1.10	66.4 ± 1.34	3.94 ± 0.69
10	3	3.56 ± 0.17	50.9 ± 0.04	2.39 ± 0.08
10	7	4.40 ± 0.21	33.1 ± 5.15	1.39 ± 0.28
10	14	4.59 ± 1.32	34.7 ± 0.50	1.03 ± 0.26
10	30	3.75 ± 0.05	35.8 ± 0.18	1.40 ± 0.01
10	60	3.98 ± 0.24	37.0 ± 1.50	1.38 ± 0.09
10	90	3.92 ± 0.29	37.9 ± 0.33	1.45 ± 0.12

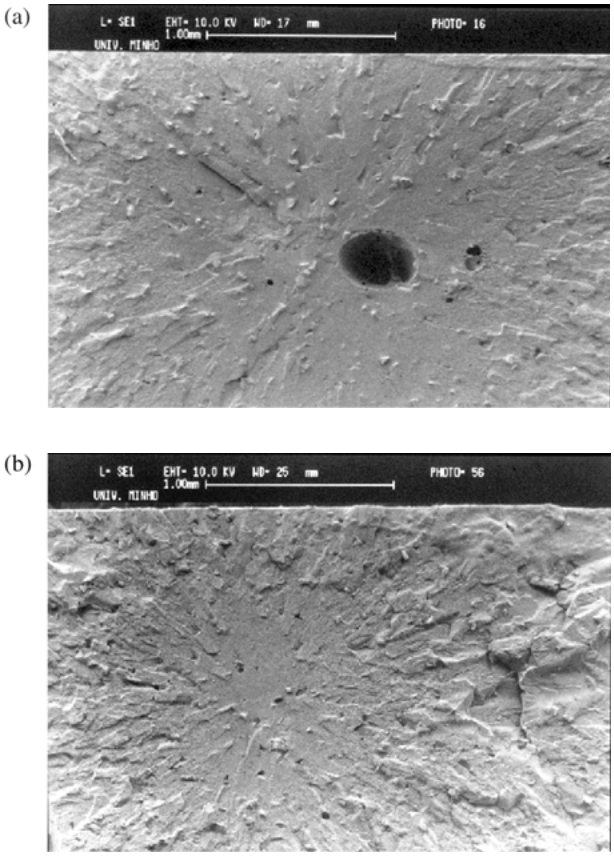

Figure 6 SEM micrograph of tensile fracture surfaces for SEVA-C 10% 45S5 composite after (a) 7 and (b) 90 days of degradation in a 0.154 M NaCl solution.

During the degradation course of the composite, a gradual load transfer to the healing tissue will occur, avoiding the stress-shielding of the surrounding healing tissues. Nevertheless, the long-term degradation behavior of the composite should be studied in future works for longer periods of degradation times.

Fig. 6 shows respectively the SEM micrographs of tensile fracture surfaces for SEVA-C composites after 7 and 90 days of degradation in an isotonic solution. Some structural changes within the matrix that result from aging in the physiological media can be observed (Fig. 6(a)). In some cases, there is a clear nucleation point for the fracture process, which is usually associated with the presence of filler particles. In Fig. 6(a), it is possible to observe a nucleation point, observed as a hole, due to the detachment of a Bioglass® particle. It is clear from both figures that all the materials exhibit a brittle fracture (Fig. 6).

4. Conclusions

SEVA-C/Bioglass® composites were successfully produced. The developed materials combine interesting mechanical properties with a degradation behavior. These composites are clearly bioactive, as it is shown by the *in vitro* bioactivity tests. The *in vitro* formation of an apatite layer on the surface of the composites is an indication of the bone-bonding ability of the developed materials. SEVA-C/Bioglass® composite systems present a high potential for a range of biomedical applications, where a balance of mechanical, bioactive and degradation properties are required, such as temporary bone replacement, fixation of fractured bones and filling of bone defects.

References

1. L. L. HENCH, in ''Bioceramics: Material Characteristics Versus in vivo Behavior'' (New York Academy of Sciences, New York, 1988) p. 54.
2. L. L. HENCH and Ö. ANDERSON, in ''An introduction to Bioceramics'' (World Scientific, Singapore, 1993) p. 41.
3. J. P. ZHONG and D. C. GREENSPAN, in ''Bioceramics 11'', New York, USA, 1998, edited by R. Z. LeGeros and J. P. LeGeros (World Scientific Pub., Singapore, 1998) p. 415.
4. M. NEO, S. KOTANI, Y. FUJITA, T. NAKAMURA and T. YAMAMURO, *J. Biomed. Mater. Res.* **26** (1992) 1419.
5. H. OONISHI, L. L. HENCH, J. WILSON, SUGIHARA, E. TSUJI, M. MATSUURA, S. KIN, T. YAMAMOTO and S. MIZOKAWA, *ibid.* **51** (2000) 37.
6. P. DUCHEYNE and Q. QIU, *Biomaterials* **20** (1999) 2287.
7. L. L. HENCH, *J. Am. Ceram. Soc.* **74** (1991) 1487.
8. W. CAO and L. L. HENCH, *Ceramics Int.* **22** (1996) 493.
9. F. MESTRAL and R. A. L. DREW, *J. Eur. Ceram. Soc.* **5** (1984) 47.
10. W. BONFIELD, J. BOWMAN and M. D. GRYNPAS, Composite Material for use in Orthopaedics, UK Patent 8032647 (1981).
11. W. BONFIELD, in ''Bioceramics 9'', Otsu, Japan, 1996, edited by T. Kokubo, T. Nakamura and F. Miyaji, (Elsevier Science, Oxford, 1996) p. 11.
12. W. BONFIELD, in ''Bioceramics 11'', New York, USA, 1998, edited by R. Z. LeGeros and J. P. LeGeros (World Scientific Pub., Singapore, 1998) p. 37.
13. W. BONFIELD, in ''Bioceramics: Material Characteristics Versus *in vivo* Behavior'' (New York Academy of Sciences, New York, 1988) p. 173.
14. G. W. HASTINGS, in ''Biodegradable Implants in Fracture Fxation'' (World Scientific, Hong Kong, 1994) p. 19.
15. M. WANG, L. L. HENCH and W. BONFIELD, *J. Biomed. Mater. Res.* **42** (1998) 577.
16. J. HUANG, M. WANG, I. REHMAN, J. KNOWLES and W. BONFIELD, in ''Bioceramics 8'', Florida, USA, 1995, edited by L. L. Hench and J. Wilson (Pergamon Press, USA, 1995) p. 389.
17. M. WANG, W. BONFIELD and L. L. HENCH, in ''Bioceramics 8'', Florida, USA, 1995, edited by L. L. Hench and J. Wilson (Pergamon Press, USA, 1995) p. 383.
18. J. C. KNOWLES and G. W. HASTINGS, *J. Mater. Sci. Mater. Med.* **4** (1993) 102.
19. N. R. BOEREE, J. DOVE, J. J. COOPER, J. KNOWLES, G. W. HASTINGS, *Biomaterials* **14** (1993) 793.
20. E. URAL, K. KESENCI, L. FAMBRI, C. MIGLIARESI and E. PISKIN, *ibid.* **21** (2000) 2147.
21. Q. LIU, J. R. DE WIJIN, D. BAKKER, M. VAN TOLEDO and C. A. VAN BLITTERSWIJK, *J. Mater. Sci. Mater. Med.* **9** (1998) 23.
22. S. VAINIONPÄÄ, P. ROKKANEN and P. TÖRMÄLÄ, *Prog. Polym. Sci.* **14** (1989) 679.
23. T. HAYASHI, *ibid.* **19** (1994) 663.
24. K. W. LEONG in ''Biodegradable Implants in Fracture Fixation'' (World Scientific, Hong Kong, 1994) pp. 45–53.
25. R. L. REIS and A. M. CUNHA, *J. Appl. Med. Polym.* **4** (2000) 1.
26. R. L. REIS, A. M. CUNHA, P. S. ALLAN and M. J. BEVIS, *J. Polym. Adv. Techn.* **16** (1997) 263.
27. R. L. REIS, A. M. CUNHA and M. J. BEVIS, *Med. Plast. Biomater.* **4** (1997) 46.
28. R. A. SOUSA, R. L. REIS, A. M. CUNHA and M. J. BEVIS, in ''Bioceramics 13'', Bologna, Italy, 2000, edited by S. Giannini and A. Moroni (Trans Tech Publications, Zurich, 2000) p. 669.
29. R. L. REIS, A. M. CUNHA, S. R. LACERDA, M. H. FERNANDES and R. N. CORREIA, in ''Bioceramics 9'', Otsuo, Japan, 1996, edited by T. Kokubo, T. Nakamura and F. Miyaji (Elsevier Science, Oxford, 1996) p. 435.

30. R. L. REIS, S. C. MENDES, Y. P. BOVELL, A. M. CUNHA, J. D. DE BRUIJN and C. A. VAN BLITTERSWIJK, in ''Proceedings of the 13th European Conference on Biomaterials'' (Göteborg, Set. 1997).
31. S. MENDES, Y. BOVELL, R. L. REIS, C. A. VAN BLITTERSWIJK and J. D. DE BRUIJN, *Biomaterials* **22** (2001) 2057.
32. M. E. GOMES, R. L. REIS, A. M. CUNHA, C. A. BLITTERSWIJK and J. D. DE BRUIJN, *ibid.* **22** (2001) 1911.
33. Y. ABE, T. KOKUBO and T. YAMAMURO, *J. Mater. Sci. Mater. Med.* **1** (1990) 233.
34. C. M. VAZ, R. L. REIS and A. M. CUNHA, *Biomaterials* **23** (2002) 629.
35. R. L. REIS and A. M. CUNHA, in ''Proceedings of Antec'98 – Plastics on my Mind'', Society of Plastics Engineers, Atlanta, USA, (1998) p. 2733.

Received 30 July
and accepted 22 October 2001